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April 12, 2000

Attorney Docket No.: 03109-017002

Box Patent Application
Assistant Commissioner for Patents
Washington, DC 20231

Presented for filing is a new divisional patent application of:

Applicant: IVAN HARGRO, JEFFREY A. HORSMAN, PETER C. RAHN, AND PETER C. VAN DAVELAAR

Title: MODULE AND METHOD FOR INTRODUCING A SAMPLE INTO A CHROMATOGRAPHY COLUMN

The prior application is assigned of record to Dyax Corporation, a Massachusetts corporation, by virtue of an assignment submitted to the Patent and Trademark Office for recording on November 4, 1998 at 9589/0351.

Enclosed are the following papers, including those required to receive a filing date under 37 CFR 1.53(b):

	Pages
Specification	10
Claims	3
Abstract	1
Declaration	2
Drawing(s)	3

Enclosures:

— Form PTO-1449, 2 pages, listing documents cited in the parent application(s). Please confirm that these have been considered in this

CERTIFICATE OF MAILING BY EXPRESS MAIL

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I hereby certify under 37 CFR §1.10 that this correspondence is being deposited with the United States Postal Service as Express Mail Post Office to Addressee with sufficient postage on the date indicated below and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

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application by returning a copy of the Form PTO-1449 with the examiner's initials.
— Preliminary amendment, 3 pages.
— Postcard.

This application is a divisional (and claims the benefit of priority under 35 USC 120) of U.S. application serial no. 09/137,278, filed August 20, 1998. The disclosure of the prior application is considered part of (and is incorporated by reference in) the disclosure of this application.

Basic filing fee	$345
Total claims in excess of 20 times $9	$0
Independent claims in excess of 3 times $39	$0
Fee for multiple dependent claims	$0
Total filing fee:	$345

A check for the filing fee is enclosed. Please apply any other required fees or any credits to deposit account 06-1050, referencing the attorney docket number shown above.

If this application is found to be incomplete, or if a telephone conference would otherwise be helpful, please call the undersigned at (617) 542-5070.

Kindly acknowledge receipt of this application by returning the enclosed postcard.

Please send all correspondence to:

WILLIAM E. BOOTH
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Boston, MA 02110-2804

Respectfully submitted,

Robert J. Silverman
Reg. No. 42,149
Enclosures
RXS/mxc
20048357.doc

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Ivan Hargro et al.. Art Unit : ---
Serial No. : -- Examiner : ---
Filed : April 12, 2000
Title : MODULE AND METHOD FOR INTRODUCING A SAMPLE INTO A CHROMATOGRAPHY COLUMN

Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Prior to examination, please amend the application as follows:

In the Specification:

On page 1, line 4, please insert the following:

--Reference to Related Applications

This application is a division of U.S. Patent Application Serial No. 09/137,278, filed August 20, 1998, the entire contents of which are hereby incorporated by reference.--

In the Claims:

Please cancel claims 1-3, and 8-15.

Please amend the claims as follows:

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4. The combination comprising

a chromatography column having a module receiving region at an inlet end thereof, and

a chromatography sample module located within said module receiving region, said module including a flow-through member having an inlet and an outlet, and chromatography media within said flow-through member.

5. (Once Amended) The [module] combination of claim 4 further comprising a sample carried on said chromatography media.

6. (Once Amended) The [module] combination of claim [1, 3 or] 5 wherein said sample has been absorbed onto said media.

7. (Once Amended) The [module] combination of claim [1, 3, or] 5 wherein said sample is dissolved in a solvent that is held within said module on said media.

Please add the following claims:

--16. The combination of claim 4, further comprising a separation media contained within said chromatography column.--

--17. The combination of claim 16 wherein said chromatography media and said separation media are made of the same material.--

--18. The combination of claim 5 wherein said sample has been dissolved in a solvent and dried onto said chromatography media.--

--19. The combination of claim 4, further comprising a sealing head connected to said module.--

--20. The combination of claim 4, further comprising a sealing head connected to said column.--

--21. The combination of claim 4 wherein said module is located entirely within said module receiving region.--

REMARKS

This is a divisional application of U.S. Patent Application Serial No. 09/137,278, filed August 20, 1998. The Applicants hereby claim priority under 35 USC §120 to U.S. Patent Application Serial No. 09/137,278.

With this Preliminary Amendment, the Applicants add a reference to the priority application, cancel claims 1-3 and 8-15, amend claims 5-7, and add claims 16-21. Support for the amendment is found in the specification and drawings as originally filed in U.S. Patent Application Serial No. 09/137,278 and also filed herewith. No new matter is added.

Applicants submit that all of the claims are in condition for allowance, which action is respectfully requested. Filed herewith is a check in payment of the excess claims fees required by the above amendments. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: April 12, 2000

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20048016.doc

Attorney's Docket No.: 03109-017002

APPLICATION

FOR

UNITED STATES LETTERS PATENT

TITLE: MODULE AND METHOD FOR INTRODUCING A SAMPLE INTO A CHROMATOGRAPHY COLUMN

APPLICANT: IVAN HARGRO, JEFFREY A. HORSMAN, PETER C. RAHN, AND PETER C. VANDAVELAAR

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Anne Roy
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Anne Roy
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MODULE AND METHOD FOR INTRODUCING A SAMPLE INTO A CHROMATOGRAPHY COLUMN

Background of the Invention

The invention relates to introducing a sample into a chromatography column.

Liquid chromatography is a technique for separating the individual compounds that exist in a subject sample. In employing the technique, the subject sample is carried in a liquid, called a mobile phase. The mobile phase carrying the subject sample is caused to migrate through a media, called a stationary phase. Different compounds will have differing rates of migration through the media, which effects the separation of the components in the subject sample. Liquid chromatography is commonly performed with reusable columns or with disposable cartridges, both of which are usually cylindrical, in which the media bed is bounded axially by porous plates, or plates containing defined flow paths, through which the mobile phase will flow. (See U.S. Pat. No. 4,250,035 to McDonald et al. and U.S. Pat. No. 5,601,708 to Leavesley)

When chemists optimize liquid chromatographic separations conditions, they may need to dissolve the sample mixture in a dissolution solvent which may be nonideal for elution. This can result in poor separation and poor recovery of desired components.

One solution to this problem is to pre-absorb the sample onto a media prior to chromatography. This involves dissolving the sample mixture in a suitable solvent and adding an amount of a dry media (usually similar to the media being used for the separation) to this solution. The dissolution solvent is then evaporated off, usually using a rotary evaporator, leaving the sample mixture dry, and absorbed to the media. The pre-absorbed media is then

placed at the head of a pre-packed glass, metal or plastic chromatography column, and the optimized chromatographic solvent would flow through the pre-absorbed media and then through the column of separation media. This method has the potential hazard of the operator coming into contact with the dry powdery media both before and after the addition of the sample. This method also can lead to poor separations and recovery.

Summary of the Invention

In one aspect, the invention features, in general, a chromatography sample module including a flow-through member having an inlet and an outlet and chromatography media within the flow-through member. A sample is added to the media, and the module, with the sample carried therein, can then be connected to a separation column.

Preferably the chromatography sample module is a tubular member that is sized to fit within the end of a chromatography column that is used for separation of the sample contained on the media in the module. Alternatively, the module can be connected to the chromatography separation column by a flow line. The sample in the dissolution solvent can be added to the sample module, and then the dissolution solvent can be evaporated. Alternatively, the sample in the dissolution solvent can be added to the sample module as a liquid without evaporation.

In another aspect the invention features a rack of sample modules arranged in an array.

Embodiments of the invention may include one or more of the following advantages. The samples can be easily introduced into separation columns. Various solvents can be used for separation and dissolution of the sample, permitting optimization of the separation procedure. Samples are easily preprocessed, and the operator is not

exposed to the media before or after adding the sample. A large number of samples can be prepared for processing at one time, facilitating the carrying out of multiple separations at one time.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims.

Brief Description of the Drawings

Fig. 1 is a schematic diagram of a chromatography system according to the invention.

Fig. 2 is a vertical sectional view of a chromatography sample module used in the Fig. 1 system.

Fig. 3 is a plan view of a rack containing a plurality of the Fig. 2 sample modules in an array.

Fig. 4 is an elevation of the Fig. 3 rack and modules.

Fig. 5 is a vertical sectional view showing the Fig. 2 sample module in position between a sealing head and a chromatography column used in the Fig. 1 system prior to assembly.

Fig. 6 is a vertical sectional view showing the Fig. 5 components in an assembled and sealed state.

Description of the Preferred Embodiments

Referring to Fig. 1, there is shown chromatography system 10 which includes a source of solvent 12, pump 14, sample module 16, liquid chromatography column 18, and sample collection vessel 20. In this system, the sample to be analyzed is preabsorbed onto media in sample module 16 prior to pumping solvent into module 16 and into chromatography column 18 to perform the separation procedure.

Referring to Fig. 2, it is seen that sample module 16 includes cylindrical plastic tube 22, porous plates 24,

26 (made of inert plastic porous frits), and chromatography media 28 (only partially shown in the figures) between porous plates 24, 26.

As appears from Figs. 5 and 6, sample module 16 is designed to fit within chromatography column 18 at the entrance thereof and to be sealably connected to the sealing head. Tube 22 is designed to fit within column 18 with minimal space between the two; in particular, there is 0.000" to 0.010" of radial clearance.

Sample module 16 can be filled with media that is the same as or is different from the media of chromatography column 18. The sample is dissolved in the required solvent and added to the top of sample module 16, where it is drawn into the media by capillary action. This dissolution solvent is then removed by placing sample module 16 in a vacuum chamber. Heat may also be applied.

After sample module 16 has dried, it can be placed directly inside separation column 18 so that the lower porous plate 26 is an in intimate contact with the surface of the separation media or with a porous plate within the separation column on top of the separation media.

Alternatively, sample module 16 can be placed in a remote tube connected by a solvent line. Alternatively, the sample can be dissolved in a separation solvent (or a weaker solvent), and added to module 16. The wet module can then be loaded into the column or into a remote tube.

Examples of the types of complex samples where this technique has particularly advantageous use include synthetic organic reaction mixtures and natural product extracts, (e.g., from fermentation broths or plants). These samples often need to be dissolved in a solvent not compatible with the optimized separation solvent. Solvents are organized according to their "solvent strength," where

hexanes have a value close to zero, and methanol has a value of 0.95. Optimized separation eluents often have a lower solvent strength; e.g., hexane:ethylacetate 1:1 has a solvent strength of 0.295. If the sample needs to be dissolved in a strong solvent such as methanol, there will be a solvent strength difference of 0.655 seen initially after loading the sample onto the column, and this will impair the separation of the sample. If the sample dissolved in methanol is instead preadsorbed to the media in the sample module and dried, the sample will not face this impairment during separation.

Referring to Figs. 3 and 4, sample modules 16 can be supplied in racks 32, and a whole rack of sample modules 16 can be efficiently prepared at one time rather than one at a time.

Figs. 5 and 6 show the placement of a module 16 in a column 18 and the sealing of the module 16 and column 18 to a sealing head used to deliver solvent. Sealing head 110 has first head piece 112, second head piece 124, intermediate head piece 128, and first and second annular elastomeric sealing members 134, 136.

First head piece 112 has body 114 with longitudinal axis 116. First head piece 112 has outwardly extending shoulder 118, and contact face 120. Part of contact face 120 has a slightly conical shape or other concavity. First head piece 112 defines flow path 122 along axis 116.

Body 114 of first head piece 112 fits slidably through central openings in second head piece 124, intermediate head piece 128, and first and second elastomeric sealing members 134, 136.

Second head piece 124 has outwardly extending compression member 146. Intermediate head piece 128 has narrow portion 148 distal from second head piece 124.

First elastomeric sealing member 134 is adjacent to both shoulder 118 and narrow portion 148 of intermediate head piece 128. Second elastomeric sealing member 136 is adjacent to both intermediate head piece 128 and second head piece 124.

The outer diameter of tube 22 of sample module 16 is sized so that tube 22 fits into column 18. The inner diameter of tube 22 is sized so that it may slidably receive shoulder 118, first elastomeric sealing member 134, and narrow portion 148 of intermediate head piece 128.

Intermediate head piece 128, second elastomeric sealing member 136, and second head piece 124 are sized to fit slidably into column 18, having chamfered edges 140, filled with chromatography separation media 142, which is bounded axially by porous plates 144.

Referring to Fig. 6 seals are formed with the apparatus by inserting sample module 16 into column 18 so that second porous plate 26 abuts first porous plate 144. Referring to Fig. 5, sealing head 110 is then inserted into column 18 and tube 22 of sample module 16, so that shoulder 118, first elastomeric sealing member 134, and narrow portion 148 are within tube 22, and contact face 120 abuts porous plate 24. Sealing head 110 extends far enough into column 18 so that second elastomeric sealing member 136 opposes the inner surface of column 18.

Downward compressive force applied to outwardly extending compression member 146 causes second head piece 124 to slide relative to first head piece 112 and transmits compressive force to second elastomeric sealing member 136, intermediate head piece 128, first elastomeric sealing member 134, shoulder 118, porous plate 24, sample module media 28, porous plate 26, porous plate 144, and separation media bed 142. The compressive force causes first and

second elastomeric sealing members 134, 136 to expand radially so that first elastomeric sealing member 134 forms a seal with tube 22, and second elastomeric sealing member 136 forms a seal with column 18.

The seals are released by relaxing or removing the downward force to second head piece 124, thereby reducing the compressive force on the components of sealing head 110 and reducing the radial expansion of elastomeric sealing members 134, 136.

Preferably, tube 22 and column 18 are made of high-density polyethylene. However, the columns may be constructed of other materials, including glass or stainless steel. Preferably, elastomeric sealing members are made of a fluorocarbon polymer, such as that sold under the trade name CHEMRAZ.

Other embodiments of the invention are within the scope of the following claims.

What is claimed is:

1. A chromatography sample module comprising a flow-through member having an inlet and an outlet, chromatography media within said flow-through member, and a sample carried on said media.

2. A chromatography sample module comprising a tubular member that is sized to fit within the end of a chromatography column, said module having an inlet and an outlet, and chromatography media within said tubular member.

3. The module of claim 2 further comprising a sample carried on said media.

4. The combination comprising a chromatography column having a module receiving region at an inlet end thereof, and a chromatography sample module located within said module receiving region, said module including a flow-through member having an inlet and an outlet, and chromatography media within said flow-through member.

5. The module of claim 4 further comprising a sample carried on said media.

6. The module of claim 1, 3 or 5 wherein said sample has been absorbed onto said media.

7. The module of claim 1, 3, or 5 wherein said sample is dissolved in a solvent that is held within said module on said media.

8. A chromatography method comprising

providing a chromatography sample module including a flow-through member having an inlet, an outlet, and chromatography media within said flow-through member,

dissolving a sample in a solvent resulting in a dissolved sample,

adding said dissolved sample to said media, and

flowing solvent into said inlet and directing the effluent from said outlet to a chromatography column.

9. The method of claim 8 further comprising evaporating said solvent from said module after said adding and prior to said flowing.

10. The method of claim 8 or 9 further comprising placing said module in said chromatography column prior to said flowing.

11. The method of claim 8 or 9 further comprising placing said module in said chromatography column prior to said flowing, and providing a seal between said module and said chromatography column prior to said flowing.

12. The method of claim 8 wherein said providing includes providing a plurality of sample modules in an array in a support structure,

each said module including a flow-through member having an inlet, an outlet, and chromatography media within said flow-through member, and

wherein said adding includes adding dissolved samples to said media in said plurality of sample modules

13. Chromatography sample preparation apparatus comprising

a plurality of chromatography sample modules, each said module including a flow-through member having an inlet, an outlet, and chromatography media within said flow-through member, and

a support structure supporting said plurality of modules.

14. The apparatus of claim 13 wherein said sample modules are adjacent to each other in said support structure.

15. The apparatus of claim 13, wherein said samples are in an array in said support structure.

MODULE AND METHOD FOR INTRODUCING A SAMPLE INTO A CHROMATOGRAPHY COLUMN

Abstract of the Disclosure

A chromatography sample module including a flow-through member having an inlet and an outlet, chromatography media within the flow-through member, and a sample carried on the media. The module can fit within a chromatography column, and a plurality of modules can be arranged in an array in a rack to facilitate processing of multiple samples.

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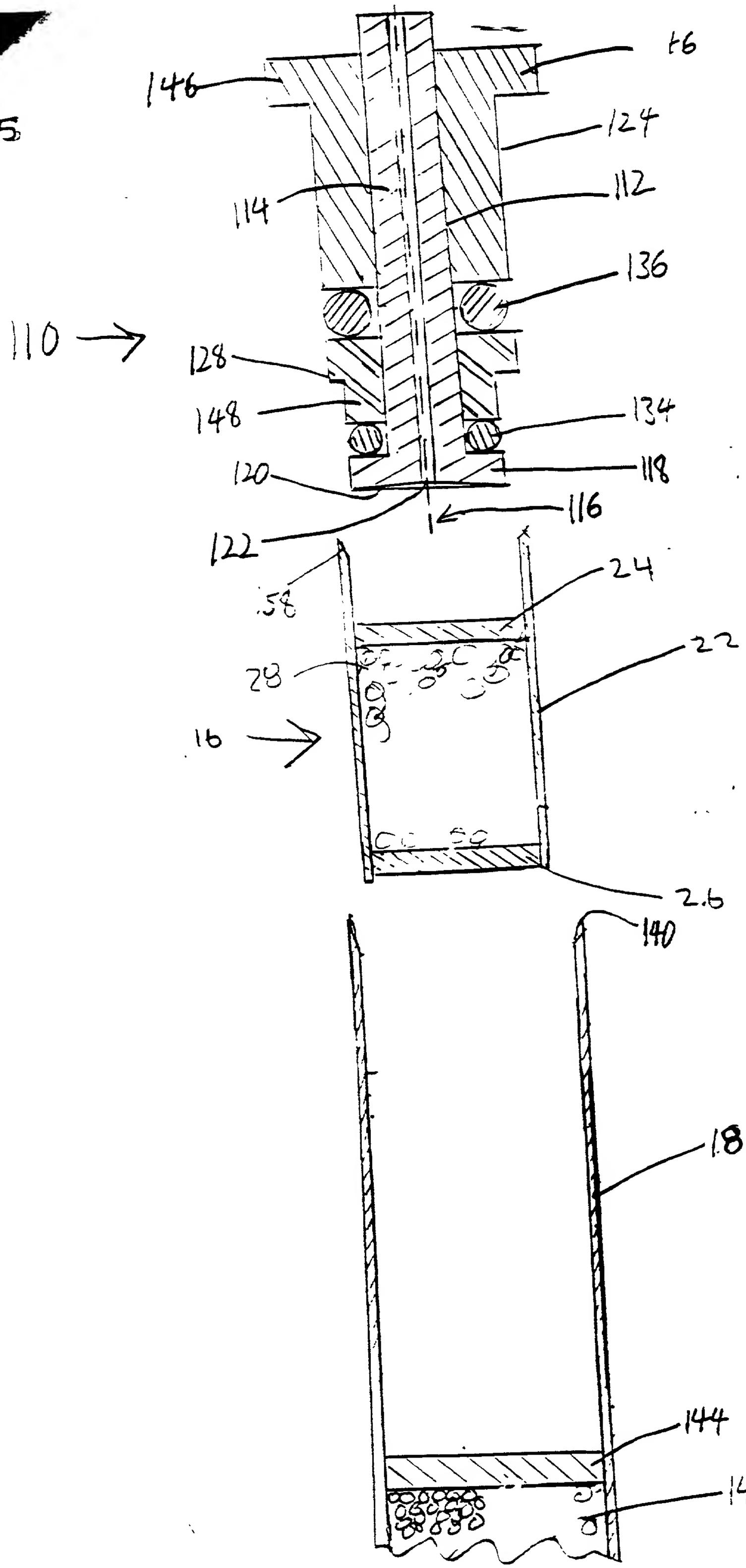
FIG. 5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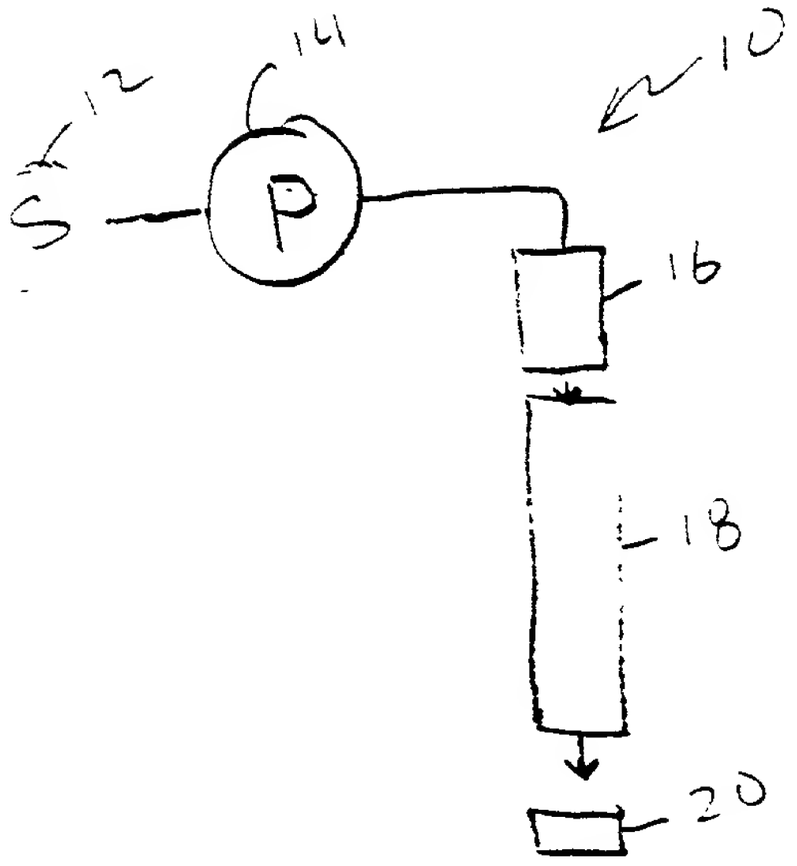


Fig. 1

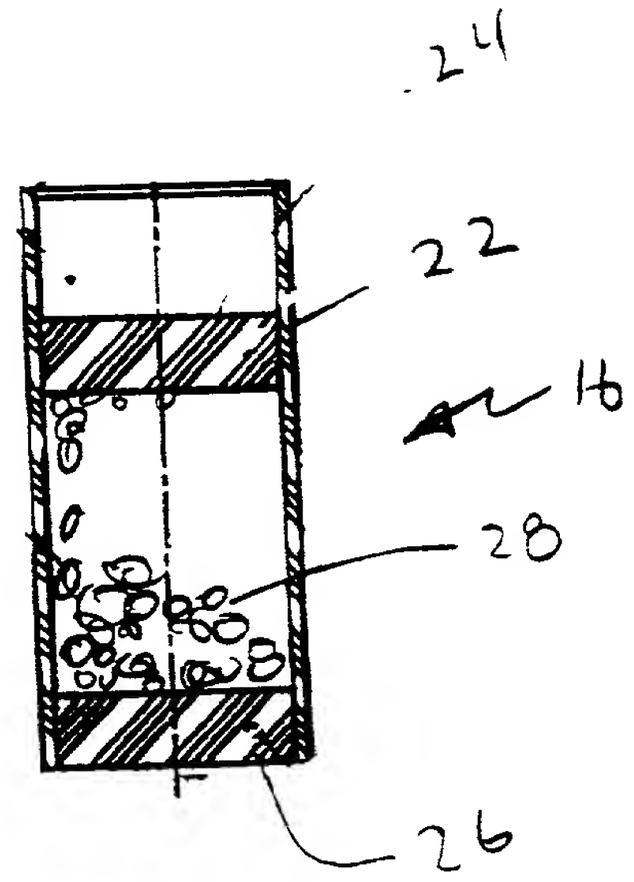


Fig. 2

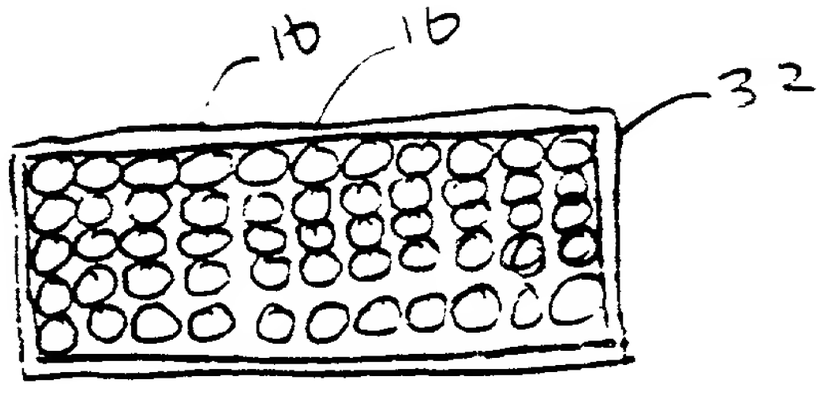


Fig. 3

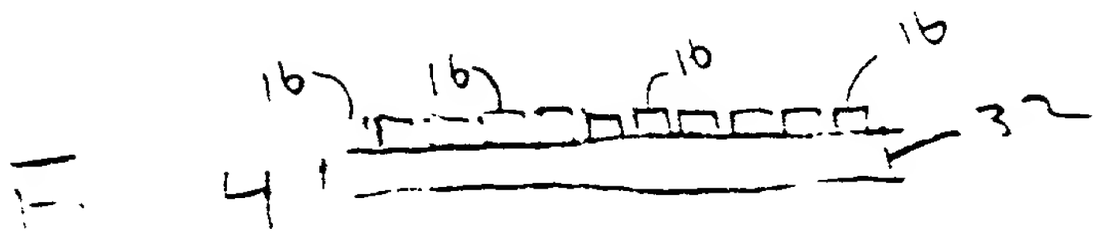
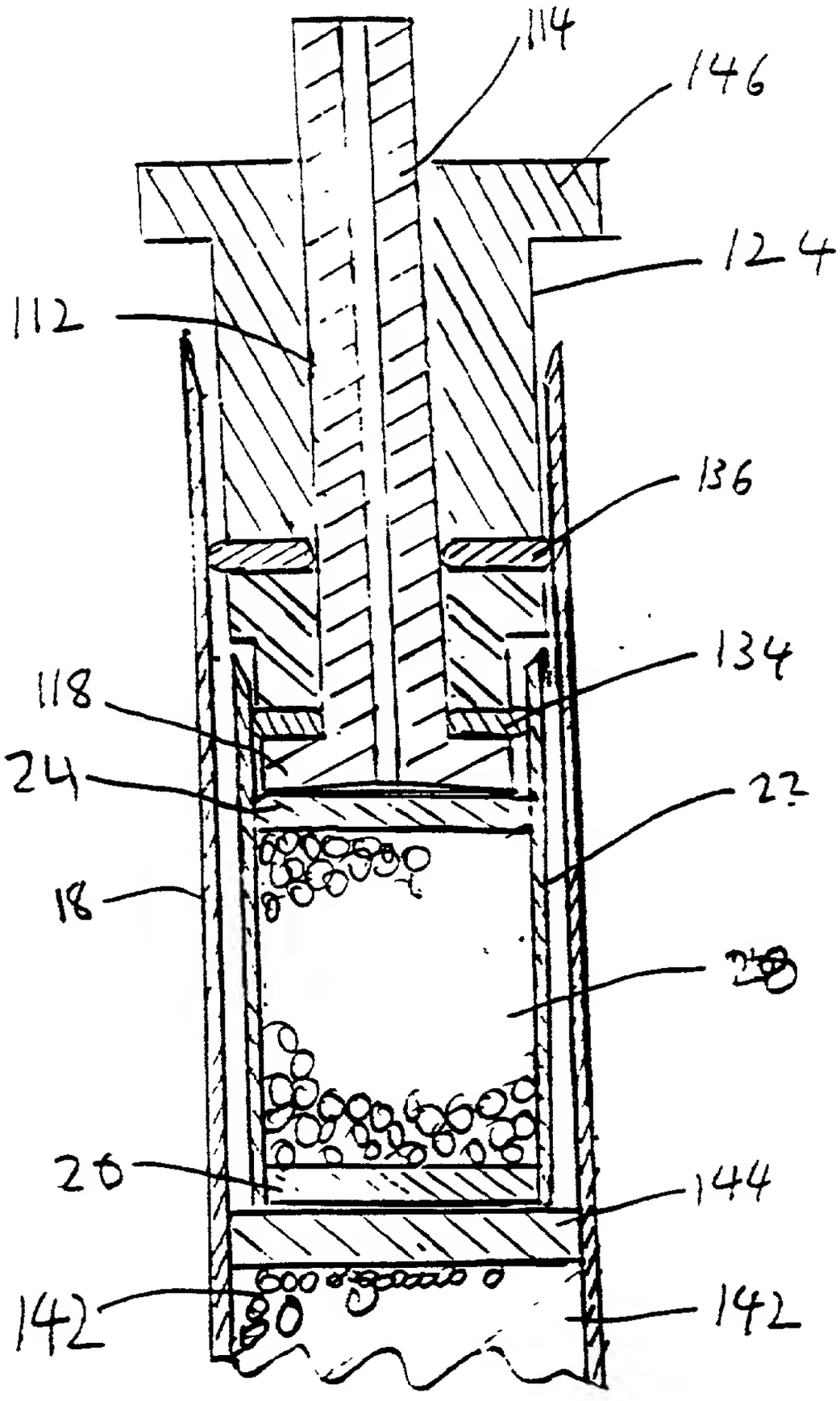


Fig. 4

FIG. 6.



PATENT
ATTORNEY DOCKET NO: 03109/017001

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believeI am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled MODULE AND METHOD FOR INTRODUCING A SAMPLE INTO A CHROMATOGRAPHY COLUMN, the specification of which

☐ is attached hereto.

X was filed on August 20, 1998 as Application Serial No. 09/137,278
and was amended on _____.

☐ was described and claimed in PCT International Application No. _____
filed on _____ and as amended under PCT Article 19 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: William E. Booth, Reg. No. 28,933

Address all telephone calls to William E. Booth at telephone number 617/542-5070.

Address all correspondence to William E. Booth, Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

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Inventor's Signature: Ivan Hargro Date: 10/15/98

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Citizen of: U.S.A.

Post Office Address: 2653 Hydraulic Road, Charlottesville, VA 22901

COMBINED DECLARATION AND POWER OF ATTORNEY CONTINUED

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